

10/588043

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Docket No. T0457-055US
Application Serial No.(not yet assigned)

In the Specification:

Please amend the specification as shown:

Please delete paragraph [086] and replace it with the following new paragraph.

[0086] *E. coli panD* open reading frame (ORF) with the start and stop codon (429 bp) was amplified from DH₅α genomic DNA using the primers 5' to 3' CCGAGCTCGACAGGGTAGAAAGGTAGA (SEQ ID NO: 3) and CCCCATGGGGATAACAATCAAGCAACC (SEQ ID NO: 4). The PCR product, cloned in pCR 2.1-TOPO vector, was verified by sequencing. A single point mutation in the clone's ORF converted Cys₂₆ to Tyr. This *panD* gene was sub-cloned in the right frame into pUC-18 vector under *lac* promoter. The pUC-*panD* vector successfully complemented an *E. coli* mutant defective in β-ala synthesis (ATCC Number AB354), confirming that the cloned gene coded for an active ADC.

Please delete paragraph [091] and replace it with the following new paragraph.

[0091] The *panD* gene was amplified from *E. coli* DH₅α genomic DNA using primers 5' to 3' TCATGATTGCGACGATGCTGCCAGG (SEQ ID NO: 5) and CAGCTGAGCAACCTGTACCGGAATCGC (SEQ ID NO: 6) primers. The *BspH* I site was introduced on the *panD* ATG start codon by the forward primer and the *Pvu* II site was introduced at the 3'end by the reverse primer. The PCR product, generated using Advantage-HF 2 PCR Kit (Clontech; Palo Alto, CA) was digested with *BspH* I and *Pvu* II and ligated directly into *Nco* I, *Pvu* II digested pET-Blue-2 vector generating pET-*panD*. Recombinant *E. coli* BL21-DE3 harboring pETB-*panD* vector or vector control, induced with IPTG, were suspended in BugBuster reagent (Novagen; Madison, WI) 5 ml•g⁻¹ wet cells, for total soluble protein extraction. Benzonase (Novagen; Madison, WI) 1 μl•ml⁻¹, β-mercaptoethanol 5 mM final concentration and a protease inhibitor cocktail as described (Rathinasabapathi *et al.*, 2001) were added. Affinity purification of the recombinant protein was performed according to the manufacturer's instructions of ProBond resin (Invitrogen; Carlsbad, CA). The sample was further purified using a 5 mL DEAE-Sepharose ion-exchange column (Sigma; St. Louis, MO). Following protein elution using a 0 to 0.3 M linear NaCl gradient, the purified ADC-His was detected using SDS-PAGE gels. Total protein was estimated by the method of Peterson (1977), and bovine serum albumin was the standard. The purified native ADC was used for raising polyclonal antibodies in rabbit according to manufacturer's protocol (Cocalico Biological, Reamstown, PA).

(g) SDS-PAGE and immunoblot analyses